NTP Research Project: West Virginia Chemical Spill

Background and Rationale

In January 2014, approximately 10,000 gallons of a liquid used to wash coal and remove impurities was spilled from a leaking tank into the Elk River in West Virginia. The spill led to the contamination of the water supply of nearly 300,000 people within nine counties in the Charleston, West Virginia metropolitan area. Reports of licorice odors at homeowner taps and hospital admittances were signs that the population was exposed to contaminated tap water. The April 2014 Centers for Disease Control and Prevention (CDC) report[1] found that one-fifth of households that received contaminated water reported health effects that they believed were related to the chemical spill. The report indicates that most of the health effects involved rashes and skin irritation; however, respiratory illnesses, nausea, and diarrhea were also reported.

The information available to date indicates that the major constituent of the spilled liquid was a technical product that contained primarily 4-methylcyclohexanemethanol (MCHM) and structurally related synthesis byproducts. In addition to this technical product ("crude MCHM"), a proprietary mixture containing predominantly dipropylene glycol phenyl ether (DiPPH) and propylene glycol phenyl ether (PPH) was also reported to be present in the leaking tank at less than 10 percent by weight. Based on material safety data sheets, several additional chemicals were likely present in the spilled liquid at lower levels, and these are noted in Table 1.

Limited toxicity data are available for the abovementioned compounds, and little to no data are available for the minor constituents of the spilled liquid. Due to the limitations of the existing database, both qualitative and quantitative uncertainty remains regarding any health risks to the exposed population. Using the limited data, CDC/Agency for Toxic Substances and Disease Registry (ATSDR) developed drinking water advisory levels of 1 part per million (ppm) for MCHM and 1.2 ppm for PPH. Summaries of existing toxicology data used to develop the drinking water advisory levels for MCHM, PPH, and DiPPH are available on the CDC website.[2,3]

In July 2014, the CDC/ATSDR nominated chemicals involved in the West Virginia Elk River Spill to the NTP for toxicological characterization. In response to this request for additional toxicology data, NTP plans to perform a number of studies of relatively short duration to provide information relevant to the potential exposures of the Charleston residents in a relatively short timeframe. The chemicals of greatest concern (e.g., those for which drinking water advisory levels were developed) will be studied in rodent toxicology models, in other model organisms, and using predictive computational modeling approaches. Chemicals of more

[1] http://www.dhhr.wv.gov/News/2014/Documents/WVCASPERReport.pdf
[2] http://www.bt.cdc.gov/chemical/MCHM/westvirginia2014/mchm.asp
[3] http://www.bt.cdc.gov/chemical/MCHM/westvirginia2014/pph.asp

limited concern (e.g., minor constituents) will be evaluated using other model organisms and computational modeling approaches.

A major focus of the toxicological characterization is the use of chemical and bioinformatics-based predictive models. These models make it possible to expeditiously: (1) evaluate effects on a broad spectrum of biological processes; (2) evaluate the need for longer-term, more comprehensive toxicology studies; and (3) provide a conservative estimate of the dose levels for where health effects might be anticipated to occur in longer-term studies. Another significant consideration in selecting the types of studies to perform is the need to assess the potential for acute exposures that result in irreversible effects. For this reason, several of the assessments will evaluate effects on fetal and early postnatal development, effects that are often irreversible. Importantly, due to the episodic nature of the exposure, the initial plan does not include the conduct of studies for the evaluation of health effects after sustained long-term exposures. The need for longer-term studies will be determined following the studies outlined here.

Specific Aims

1. Evaluate the teratogenic, immunotoxic, and genotoxic potential of MCHM.

2. Identify sensitive, biological effects of the spill chemicals to determine if there is the potential for low-dose, biological effects in humans and to provide additional information about no adverse effect levels.

3. Use efficient, medium- and high-throughput testing methods to derive information for predicting the qualitative and quantitative toxicological properties of all chemicals spilled in the Elk River.

Planned NTP Studies

The NTP studies that will be performed on each chemical are shown in Table 1. In this table, the resource-intensive nature of the assessment decreases from left to right (i.e., the teratology assessment is the most time and resource-intensive study).

Table 1. NTP Studies on Elk River Spill Chemicals and Structurally Related Compounds

	Studies								
Test Article [Abbreviation, CAS Number]	**Rat Prenatal Developmental Toxicity Study (Teratology)**	**Mouse Dermal Irritation and Hypersensitivity**	**5-Day Rat Toxicogenomic**	**Bacterial Mutagenicity**	**Zebrafish Developmental**	**Nematode Toxicity**	**High Throughput Screening**	**Structure Activity Relationship (SAR) Analysis**	**Notes**
4-Methylcyclohexanemethanol [MCHM, 34885-03-5]	X	X	X	X	X	X	X	X	a
Dipropylene glycol phenyl ether [DiPPH, 51730-94-0]			X	X	X	X		X	b
Propylene glycol phenyl ether [PPH, 770-35-4]			X	X	X	X	X	X	b
1,4-Cyclohexanedimethanol [CHDM; 105-08-8]				X	X	X	X	X	b
2-Methylcyclohexanemethanol [2MCHM, 2105-40-0]				X	X	X		X	b
4-(Methoxymethyl)cyclohexanemethanol [MMCHM, 98955-27-2]				X	X	X		X	b
4-Methylcyclohexanecarboxylic acid [4331-54-8]					X	X		X	c
Cyclohexanemethanol, 4-[(ethenyloxy)methyl]- [114651-37-5]					X	X	X	X	c
Cyclohexanemethanol, alpha,alpha,4-trimethyl- [498-81-7]					X	X		X	c
Dimethyl 1,4-cyclohexanedicarboxylate [DMCHDC, 94-60-0]				X	X	X	X	X	b
Methyl 4-methylcyclohexanecarboxylate [MMCHC, 51181-40-9]				X	X	X		X	b
Phenoxyisopropanol [4169-04-4]					X	X	X	X	c
Technical product [“crude MCHM”]		X	X	X	X	X			d

[a]Major (>50% of the spilled chemical mixture) constituent of the spilled liquid
[b]Minor (<10% of the spilled chemical mixture) constituent of the spilled liquid
[c]Not a component of the spilled liquid, but included because the compound is structurally related to MCHM or PPH
[d]The commercial product present in the leaking tank; a mixture of MCHM, MMCHM, MMCHC, DMCHDC, CHDM, and methanol

Rat Prenatal Developmental Toxicity (Teratology) Study. A potential concern of acute exposures is the effect on the developing fetus. The occurrence of birth defects often depends upon the developmental stage of the fetus at the time of chemical exposure. The goal of a teratology study is to determine if a chemical has the potential to produce adverse effects on fetal survival or fetal development, and/or cause birth defects at any fetal development stage. This study will address, in part, concern over spill-related exposures to pregnant women in the

Charleston area. MCHM was selected for this assessment because it is the primary chemical in the spilled liquid and the effects of MCHM on fetal development are unknown.

Mouse Dermal Irritation and Hypersensitivity Study. A combined local lymph node/irritancy assay evaluates the ability of a drug or chemical to cause skin inflammation by directly damaging cells and causing irritation, or by inducing an immune response known as allergic hypersensitivity or contact allergy. This study will focus on MCHM because it is the major constituent of the spilled liquid. Available data suggest that MCHM does not cause hypersensitivity, but is a known irritant; however, there is very little information about its potency in relation to its irritant effects. This study will provide information on the dose level where exposure to MCHM does not cause irritancy (a "no effect" level). In addition to purified MCHM, crude MCHM will also be assessed to determine if additional constituents of the mixture might alter the toxicological properties of MCHM.

5-Day Rat Toxicogenomic Study. A 5-day toxicogenomic study is intended to identify subtle effects of a chemical on molecular processes in the liver and kidney and enable the collection of data on endpoints indicative of a toxic effect in blood (e.g., clinical chemistry, hematology) and damage to DNA (genetic toxicity). In addition, through the use of computational approaches and information from similar studies on a wide range of other chemicals, it is possible to identify disease-related processes that may be affected in humans by a chemical. This study will be performed using a wide range of doses of the spill chemicals to facilitate the identification of biological processes that are most sensitive to their effects. In addition, data will be used to identify a "no effect" dose level, a level at which there would be no anticipated adverse effect from exposure. MCHM, PPH, and DiPPH were selected because they are the chemicals for which drinking water advisory levels were developed. Crude MCHM is included in these studies to determine if additional constituents of the technical product might alter the biological effects caused by MCHM.

Bacterial Mutagenicity Studies. These are a set of short-term, in vitro tests to evaluate DNA damage (genetic toxicity) in the bacteria *S. typhimurium* and *E. coli* caused by exposure to a chemical. The results of these tests correlate well with the outcome of studies of carcinogenicity in rodent models.[4] DNA mutagenesis, or the process where genetic information in an organism is changed, is an irreversible process and can occur following acute exposure to a chemical. The limited data that are available on a subset of the chemicals of interest and structure activity relationships suggest the spill chemicals have a low potential to cause genetic toxicity. These studies will evaluate all major and minor components of the spilled material and the crude MCHM technical product listed in Table 1 for their ability to cause mutations in several bacterial species and strains.

Zebrafish (*Danio rerio*) Developmental Study. Studies in zebrafish provide a rapid screen for identifying developmental effects in a vertebrate model system, and this model organism has been extensively used in basic biomedical research for many years. More than 20 teratological

[4] http://ntp.niehs.nih.gov/go/9407

and behavioral measurements are captured at different developmental stages making it possible to identify a range of effects that a chemical may have on development. All chemicals in Table 1 will be evaluated using this assay.

Nematode (*Caenorhabditis elegans*) Toxicity Study. The *C. elegans* (nematode worm) is used as a model organism to rapidly screen for and identify a variety of effects at different life stages. Four primary measurements (feeding, growth, reproduction, and locomotion) are evaluated in the life cycle toxicity assessment. All chemicals in Table 1 will be evaluated in this assay to provide insight into their toxicological potency relative to many other chemicals the NTP has studied in this model system previously.

High Throughput Screening (HTS) Studies. The federal Tox21 HTS Program[5] is evaluating 10,000 chemicals in cellular and molecular-based assays to identify potential toxicological/biological properties. Four of the spill chemicals (MCHM, CHDM, DMCHDC, and PPH) are included in the 10,000 chemicals library and, therefore, HTS data are available for these chemicals. Review of the available data will determine if a subset of the spilled chemicals (noted in Table 1) have biological effects related to specific, well-studied toxicological processes and enable comparison of their HTS results to other chemicals with known toxicities.

Structure-Activity Relationships (SAR) Analysis. SAR analysis is a computational assessment that uses a chemical's structure to predict its toxicological or biological properties. This analysis will evaluate the chemicals in Table 1 using a variety of SAR software platforms across a wide range of toxicological endpoints.

Significance and Expected Outcomes

The short-term studies outlined here will provide information for addressing critical toxicological concerns surrounding the chemicals spilled into the Elk River. The project leverages emerging technologies to provide a large body of data that can be used by federal and state agencies for risk assessment and to determine if longer-term, resource-intensive definitive studies need to be performed. It is anticipated that the teratology study will be reported in 9-12 months, and results from the less-resource intensive studies will be available in a 3-6 month timeframe.

[5] http://ntp.niehs.nih.gov/results/hts/index.html